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► To cite this version:

Anne Thebault, Pauline Kooh, Vasco Cadavez, Ursula Gonzales-Barron, Isabelle Villena. Risk factors for sporadic toxoplasmosis: A systematic review and meta-analysis. *Microbial Risk Analysis*, 2021, 17, pp.100133. 10.1016/j.mran.2020.100133 . hal-03086254

HAL Id: hal-03086254

<https://hal.univ-reims.fr/hal-03086254>

Submitted on 9 May 2023

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1 **Risk factors for sporadic toxoplasmosis: a systematic review and meta-** 2 **analysis**

3
4

5 **Short Title:** Meta-analysis on risk factors for sporadic toxoplasmosis

6 **Abstract**

7 Toxoplasmosis is considered as the most prevalent parasitic zoonotic infection worldwide.
8 The parasitic cycle is mostly known, but the relative contribution of different sources and
9 pathways of transmission was not previously studied by a meta-analysis. A systematic review
10 and a meta-analysis of case-control, cohort, and cross-sectional studies were performed to
11 determine the main risk factors associated with sporadic *Toxoplasma* infection. Suitable
12 scientific articles were identified through a systematic literature search and subjected to a
13 methodological quality assessment. Mixed-effects meta-analysis models were adjusted by
14 population type – children, mixed population, and pregnant women – to appropriate data
15 partitions. 187 primary studies passed the quality assessment stage, investigating risk factors
16 for sporadic infection with *Toxoplasma gondii* conducted between 1983 and 2016. Cases were
17 defined by serology.

18

19 The meta-analysis of *Toxoplasma* sporadic infections revealed the significance of
20 transmission by environmental factors such as contact with soil and contact with animals, in
21 particular cats. The consumption of raw or undercooked meat and unwashed vegetables
22 significantly increased the odds of acquiring the disease. Shellfish and raw milk were
23 identified as significant sources of toxoplasmosis. Almost all meat categories were identified
24 as risk factors: pork, poultry, beef, processed meat, lamb, and game meat. Contaminated
25 drinking water may play a role in the acquisition of infection. Moreover, the lack of hygiene
26 in preparing food was identified as a risk factor. A significant risk factor for pregnant women
27 is traveling abroad. Lastly, blood transfusion (in pregnant women) and immunocompromised
28 conditions were found associated with positive serology. The broad definition of exposures
29 and the use of serology for the case definition are the main limitations for the interpretation of
30 the results of this meta-analysis. The transmission pathways require further investigations
31 using longitudinal studies and subtyping approaches.

32
33

34 1. Introduction

35

36 *Toxoplasma gondii*, an obligate protozoan parasite of the *Apicomplexa* phylum, is a
37 worldwide parasite that can infect humans and a large range of warm-blooded vertebrates.
38 Three major clonal lineages (type I to III) differ in pathogenicity and prevalence around the
39 world, with genotype II dominating in congenital toxoplasmosis cases in Europe and the USA
40 (Hosseini *et al.*, 2019). The disease is generally benign, but some severe or life-threatening
41 effects can occur in children (Dunn *et al.*, 1999), when the transmission is congenital, and in
42 immunocompromised patients (Robert-Gangneux and Darde, 2012). Since the conventional
43 designation assumed this clonal population structure, other genotypes have been identified
44 worldwide and termed as “atypical” or “exotic” (Dardé *et al.*, 2014). Among these, highly
45 virulent strains circulating mainly in South America have been responsible for severe cases in
46 immunocompetent people (Carme *et al.*, 2009). Approximately 30% of the human population
47 is considered infected (Montoya and Liesenfeld, 2004). Serological tests are usually used to
48 detect the infection, with detection of anti-*T. gondii* specific IgG and/or IgM antibodies
49 (Montoya, 2002). *T. gondii* is globally distributed and results in a high public health impact.
50 The Global Disease Burden 2015 Study estimated that foodborne toxoplasmosis was
51 responsible for 10.3 million (95% UI 7.40–14.9 million) cases in 2010, and 825,000 DALYs
52 (95% UI 561,000–1.26 million) DALYs (Torgerson *et al.*, 2015).

53

54 The parasitic cycle of toxoplasmosis is complex. During its primary infection, the cat (or
55 other felines), the definitive host, excretes parasites (oocyst form) in its stool. Excretion in
56 cats is limited in time (about two to three weeks) until immunity is established. Oocysts can
57 contaminate the environment: the soil, water, and therefore shellfish that filter water and plant
58 products directly or via irrigation water. Excreted oocysts are not infectious and become
59 infective after sporulation, after few days in the environment depending on climate
60 conditions, and become infectious with long resistance in environmental conditions (oocysts
61 can survive for long periods, up to years, in a favorable environment). The remarkable
62 resistance of the oocyst wall enables the dissemination of *T. gondii* through watersheds and
63 ecosystems, and long-term persistence in diverse foods such as shellfish and fresh produce
64 (Shapiro *et al.*, 2019). Humans and all warm-blooded mammals are infected through the
65 environment or food. Parasites encyst in all tissues, especially striated muscles and the brain.
66 These cysts persist throughout life and can be a source of contamination of new hosts through
67 meat ingestion (carnivorism) (Tenter *et al.*, 2000).

68

69 *T. gondii* exposure to humans may have multiple origins, and the prevalence is high (and
70 protective for pregnant women). So, numerous epidemiological studies investigate the main
71 transmission pathways of sporadic *T. gondii* infection by serological studies. A systematic
72 review of outbreaks was recently published (Meireles *et al.*, 2015), still a systematic review
73 and a meta-analysis of case-control, cohort, and cross-sectional studies have to be performed
74 to determine the main risk factors associated with sporadic *T. gondii* infection.
75 Characterization of risk factors of *T. gondii* could contribute to identify recommendations for
76 susceptible populations such as pregnant women or immunocompromised patients. The
77 objective of this meta-analysis is to summarize the evidence on risk factors for sporadic *T.*
78 *gondii* infection regardless of the country of origin from relevant scientific information
79 contained in epidemiological case-control/cohort/cross-sectional studies.

80

81 **2. Material and methods**

82 The protocol of the systematic review and the meta-analysis model are described in depth in
83 the methodological paper of this issue (Gonzales–Barron *et al.*, 2019).

84

85 **2.1 Systematic review**

86 The literature search was conducted between March 2017 and December 2017 using a
87 combination of keywords related to (1) Toxoplasma OR Toxoplasmosis, (2) “case-control”
88 OR “risk factor” OR cohort (3) infection OR disease, joined by the logical connector AND.
89 Relevant studies were identified from five bibliographic search engines, Science Direct,
90 PubMed, Scielo, ISI Web of Science and Scopus. No restrictions were defined for the year of
91 the study or type of publication. The search was limited to the languages English, French,
92 Portuguese and Spanish (Gonzales–Barron *et al.*, 2019).

93 Each reference record was screened for relevance for inclusion in the meta-analysis study, and
94 subsequently, the methodological quality of the “candidate” studies was assessed using pre-
95 set quality criteria comprising (1) appropriate selection of the controls; (2) adjustment to
96 correct for confounders, (3) comparability between cases and controls, (4) acceptable
97 responses rates for the exposed and control groups; (5) Data analysis appropriate to the study
98 design; (6) provision of Odd ratio (OR) with confidence interval or p-value; or provision of
99 sufficient data to calculate ORs; overall quality of the study (Gonzales-Barron *et al.*, 2019).

100 Primary studies that passed the screening for relevance were marked as having a potential for
101 bias if they failed to meet at least one of the methodological quality assessment criteria.

102

103 Data from primary studies were extracted using a standardized spreadsheet. Data extracted
104 included the relevant study characteristics (location, time period, population, case definition,
105 design, sample size of the groups, type of model, etc.), the categorized risk factors, the setting,
106 the handling practices and the outcome of the study, odds-ratios (ORs). A data categorization
107 scheme was established to hierarchically group the risk factors into travel, host-specific
108 factors, and pathways of exposure (i.e., person-to-person, animal, environment and, food
109 routes – refer to Gonzales-Barron *et al.*, 2019). In addition to the standard risk factors, the
110 class “Hygiene” (e.g. “no handwashing after toilet”, “poor hygiene habits”) was also used.
111 Host-specific factors includes blood transfusion.

112

113 Considering the parasitic cycle of *T. gondii*, specific partitions were made to investigate more
114 deeply risk factors. The food class “Other red meats” were stratified into lamb, mutton, goat,
115 boar, and venison/horse meat. The route “contact with cats” was also added within “contact
116 with pets”. Sexual transmission was found irrelevant and was excluded from the analysis, as
117 humans are not a reservoir of *Toxoplasma*, nor involved in fecal-oral transmission. The
118 variable “Population” was stratified into mixed (adults, or age not defined), pregnant women,
119 and children (under 16 years old). Susceptible population (HIV infection, AIDS, new-borns,
120 liver diseases, chronic kidney diseases, solid organ transplants, mental illnesses, and
121 neurologic diseases) was excluded at the beginning of the study. The acquisition of
122 *Toxoplasma* could be far before the illness (congenital for new-borns) and
123 immunosuppression is by example, a well-known factor of reactivation of a pre-existing
124 infection. Therefore, it was not relevant to study risk factors of acquiring *Toxoplasma* on such
125 populations. Host-specific factors were reduced to immunosuppressive factors that could be
126 linked to a causal pathway of reactivation. Other factors were found irrelevant and excluded
127 because those factors cannot explain the acquisition of the infection (*i.e.* mental illnesses).

128

129 **2.2 Data synthesis**

130 The full meta-analytical data was first described using basic statistics. Next, data was
131 partitioned into subsets of categories of risk factors (“data partitions”), such as travel, host-
132 specific factors including transmission pathways related to blood transfusion , animal contact,
133 environmental exposures, and food vehicles. Meta-analysis models were then fitted to each of
134 the data partitions, with subgroup class that depends of data partition. For instance, if the data
135 partition was travel, the meta-regression was taking into account the subgroup classes: inside,

136 abroad, any (Gonzales-Barron et al., 2019). The meta-analytical models were fitted separately
137 by population type. For some food classes, the effects of handling (i.e., eating raw,
138 undercooked) and setting (i.e., eating out) on the overall OR were assessed by the calculation
139 of the ratio of the mean OR when food is mishandled to the base OR (Gonzales-Barron *et al.*,
140 2019).

141
142 The statistical analysis was designed to assess the effect of the geographical region, the study
143 period, and the analysis type (univariate/multivariate) on the final result. The objective of the
144 region-specific meta-analysis was to inform the decision on whether the geographical regions
145 were to be maintained for the subsequent pooling of ORs. Geographical regions (Asia, North
146 America, South America, Africa, Europe, Oceania) were removed from a particular meta-
147 analysis partition when their ORs, for this partition, were different from those associated with
148 the other regions or if only less than 3 ORs represented the region (Gonzales-Barron *et al.*,
149 2019).

150 All meta-analysis models were substantially weighted random-effects linear regression
151 models. Once a meta-analysis model was fitted, influential diagnostics statistics were applied
152 to remove any influential observation originating from studies marked as having potential-for-
153 bias. Publication bias was assessed by funnel plots and statistical tests (Gonzales-Barron *et*
154 *al.*, 2019) and a statistical test investigating the effect of the study sample size on ORs (Tables
155 2, 3 and 4) (Gonzalez-Barron *et al.*, 2019). Heterogeneity between studies was assessed by
156 different indicators such as the between-study variability (τ^2), the QE test investigating
157 residual heterogeneity, the variance of residuals, and the intra-class correlation I^2 (Gonzales-
158 Barron *et al.*, 2019).

159 All analyses were produced in the R software (R Development Core Team, 2008)
160 implemented with the *metafor* package (Viechtbauer, 2010).

161

162 **3. Results**

163 **3.1 Descriptive statistics**

164 The quality assessment stage was passed by 213 primary studies investigating risk factors for
165 sporadic infection with *T. gondii*, which were conducted between 1983 and 2016 (80.5% after
166 2000). Excluding susceptible populations other than pregnant women, and some risk factors
167 (see above), 187 publications were retained for meta-analysis (Figure 1 and Appendix 1).
168 Primary studies investigated risk factors in different types of population, namely children (16
169 studies), mixed population (98 studies), and pregnant women (76 studies) (some of them also

170 studied other populations) (Appendix 2). The majority of publications concerns, in descending
171 order, South America (32.5%), Asia (30%), Africa (17.5%), Europe (15%), North America
172 (4%), and Oceania (1%). All publications produced 2050 ORs.

173

174 Toxoplasmosis is generally asymptomatic, so only the presence of antibodies indicates past
175 infection. Symptomatic forms are observed mainly in children infected by congenital
176 transmission (infection occurring during pregnancy in mothers), in immunocompromised
177 people by reactivation of their infection, and more rarely in immunocompetent people. In
178 primary studies, the toxoplasmosis infection was diagnosed by routine antibody screening for
179 *T. gondii* IgG and/or IgM antibodies. The target populations considered are the mixed
180 population (930 OR), pregnant women (841 OR), and children (185 OR). During the
181 methodological quality assessment, fourteen studies were marked as being possibly affected
182 by bias (Appendix 2). Potential for selection bias was assigned to thirteen studies whose
183 population groups were believed to have a stronger exposure to *Toxoplasma gondii* infection,
184 such as waste pickers and waste workers (Alvarado-Esquivel *et al.*, 2008; Alvarado-Esquivel
185 *et al.*, 2010), livestock and abattoir workers (Adeyisum *et al.*, 2011; Alvarado-Esquivel *et al.*,
186 2011; Alvarado-Esquivel *et al.*, 2014b), agricultural workers or rural people living in poverty
187 (Alvarado-Esquivel *et al.*, 2013; Cavalcante *et al.*, 2006; Doni *et al.*, 2015; Rostami *et al.*,
188 2016), veterinary practitioners (Brandon-Mong *et al.*, 2015), inmates (Alvarado-Esquivel *et al.*
189 *et al.*, 2014a; Sari *et al.*, 2015), and female patients with miscarriages (Tammam *et al.*, 2013).
190 The rationale for assigning a potential-for-bias status to the association measures extracted
191 from Gyang *et al.* (2015) related to the statistical approach employed, which was a log-
192 binomial model producing prevalence ratio estimates. As explained in Gonzales-Barron *et al.*
193 (2019), potentially-biased individual ORs were removed only if their influence on the pooled
194 OR estimates was significant, as assessed by the Cook's distance. The risk factors studied
195 concerned the following routes of exposure: host-specific factors (immunocompromised
196 conditions and blood transfusion) (71 OR), lack of personal hygiene (10 OR), travel (21 OR),
197 environmental transmission (476 OR), contact with animals (pets including cats, farm
198 animals, wildlife (511 OR), and food (867 OR).

199

200 **3.2 Meta-analysis results**

201 For every data partition, the meta-analyzed risk factors are presented in summary tables only
202 when significant (Tables 1, 2 and 3). Pooled ORs were considered as significant when the
203 lower bound of the 95% CI was equal or greater than 1. Non-significant results for main risk

204 factors are given in Appendix 3. According to this meta-analysis, travel abroad is a significant
205 risk factor for acquiring positive *Toxoplasma* serology for pregnant women (pooled
206 OR=1.878; 95% CI: 1.284 - 2.746) (Table 1). However, it was non-significant for mixed
207 population, whatever the countries of origin or destination.

208

209 Immunocompromised conditions, such as cancer, immunosuppressive treatment or HIV
210 infection, were found to be associated with positive *Toxoplasma* serology in the mixed
211 population (14 publications, pooled OR=2.407; 95% CI: 1.483- 3.909). However, it was non-
212 significant for the pregnant population, with seven publications. Blood transfusion was a
213 significant risk factor for acquiring positive *Toxoplasma* serology in pregnant women (pooled
214 OR=1.785; 95% CI; 1.031 - 3.089) (Forest plot in Figure 2). For the mixed population, blood
215 transfusion did not reach significance (OR=1.368; 95% CI: 0.793 - 2.358). Poor personal
216 hygiene (i.e. “poor hands hygiene”) was jointly analyzed for mixed and children populations,
217 and was found significantly associated with positive *Toxoplasma* serology (pooled OR=2.023;
218 95% CI: 1.693 - 2.416).

219

220 Regarding the animal contact pathways, significant associations were found for contact with
221 animals in the mixed, pregnant, and children populations. Occupational contact (raising
222 animals, or contact with animal products) were significant risk factors in both mixed (pooled
223 OR=2.035) and pregnant populations (pooled OR=1.557). Similarly, contact with farm
224 animals was also found significant in the mixed population (pooled OR=1.482; 95% CI: 1.099
225 - 1.998). Contact with ~~(wild animals)~~ flies/rodents was found significant for all three
226 subpopulations. However, it is a heterogeneous category rather reflective of a relative lack of
227 hygienic conditions of living, with rats or flies inside or near the house (pooled OR from
228 1.435 to 1.534) (Figure 3). For all the populations studied (mixed, pregnant, or children
229 population), contact with a pet (Table 1), and in particular with cats (Table 2), was a
230 significant risk factor with pooled ORs between 1.631 and 1.711 (Table 2).

231

232 For the environmental pathways, consumption of untreated drinking water, farm environment
233 (“living on farm”), contact with wastewater (including “lack of toilets”) and contact with soil
234 (such as gardening, playground) were significantly associated with positive *Toxoplasma*
235 serology for the three target populations (except in children population for playground) (Table
236 1). For the mixed population, 22 ORs coming from Africa region were excluded, due to the
237 high level of OR, concerning untreated drinking water (9), farm environment (6), and

238 playground (7). Of course, considering them is not changing the significance of results
239 concerning those relevant categories (results not shown).

240 For the children population, one OR from an Oceanian study was excluded, because it was
241 isolated. Six ORs coming from Africa were also excluded, describing exposure to drinking
242 unboiled water and playground, because of the low values of their OR, in comparison with
243 other regions. For the children population, the contact with soil and garden (“playground”)
244 was close to reaching significance (pooled OR=1.138; 95% CI: 0.969 - 1.335).

245

246 The meta-analysis on food consumption pathways revealed significant associations with meat
247 for the mixed population (pooled OR=1.761; 95% CI: 1.570 - 1.974) and pregnant women
248 (pooled OR=1.960; 95% CI: 1.472 - 2.610) (Table 1). By contrast, in the children population
249 from six publications from South America and Africa, the overall OR associated with meat
250 was not significant (pooled OR=1.329; 95% CI: 0.891 - 1.985; Appendix 3). For the mixed
251 population, almost all meat matrices were identified as risk factors: pork meat (pooled OR=
252 2.114; 95% CI: 1.411 - 3.169), poultry (pooled OR=1.623; 95% CI: 1.147 - 2.297), beef
253 (pooled OR=1.635; 95% CI: 1.170 - 2.285), processed meat like salami or sausages (pooled
254 OR=1.365; 95% CI: 1.075 - 1.733), other red meat (goat, mutton or lamb) (pooled OR=
255 1.897; 95% CI: 1.480 - 2.431) and other types of meat (game or undercooked meat) (pooled
256 OR=1.734; 95% CI: 1.544 - 1.947]) (Table 2). For this population, when it was possible, more
257 precise items could be analyzed such as wild boar meat (pooled OR=2.487; 95% CI: 1.814 -
258 3.409), lamb meat (pooled OR=2.404; 95% CI: 1.189 - 4.859), goat meat (pooled OR=1.667;
259 95% CI: 1.212 - 2.294), and other types of red meat (such as venison, ram and horse meat)
260 (pooled OR=1.566; 95% CI: 1.221 - 2.008) (Table 2).

261

262 In pregnant women, significant sources were beef (pooled OR=2.052; 95% CI: 1.576 - 2.672),
263 other red meat (goat, mutton or lamb) (pooled OR=1.822; 95% CI: 1.279 - 2.595), poultry
264 (pooled OR=1.514; 95% CI: 1.130 - 2.028), processed meat, like cured meat, sausages and
265 salami (pooled OR=1.532; 95% CI: 1.201 - 1.953), and other meat such as undercooked or
266 raw meat (most often in this category) and game meat (sometimes) (pooled OR=1.592; 95%
267 CI: 1.354 - 1.871) (Table 2). The pork consumption was not found significant for pregnant
268 women with a pooled OR=1.04 (95% CI: 0.780 - 1.387). Within the red meat category
269 (including lamb and mutton/sheep), only the consumption of lamb was found significant
270 (pooled OR=1.832; 95% CI: 1.148 - 2.922; Table 2). In children, meat, dairy products, and

271 produce consumption were not found significantly associated with positive *Toxoplasma*
272 serology.

273

274 The consumption of produce was associated with positive *Toxoplasma* serology for the mixed
275 population (pooled OR=1.872; 95% CI: 1.539 - 2.276) and pregnant women (pooled
276 OR=1.651; 95% CI: 1.267 - 2.151) (Table 1). Within produce, only vegetables were identified
277 as a risk factor for the mixed population (pooled OR=1.866; 95% CI: 1.491 - 2.335) and
278 pregnant women (pooled OR=1.372; 95% CI: 1.198 - 1.571) (Table 2). The consumption of
279 seafood in the mixed population (not studied in pregnant nor in children population) (Table1),
280 in particular shellfish (“mollusks”) (Table 2), was found in association with positive
281 *Toxoplasma* serology (pooled OR=1.917; 95% CI: 1.395 - 2.636) (Figure 5). Nevertheless,
282 the fish consumption was not found significant associated with toxoplasmosis.

283

284 The consumption of eggs was not found to be a determinant of positive *Toxoplasma* serology
285 either in the mixed or in the pregnant population. Dairy products (mainly raw milk) were
286 significant risk factors for the mixed population (pooled OR=1.563; 95% CI: 1.298 - 1.882)
287 (Figure 4) and pregnant women (pooled OR=1.521; 95% CI: 1.116 - 2.073) (Table 1). The
288 consumption of raw milk or raw milk cheese in comparison with pasteurized dairy products
289 significantly increases the risk by a factor of 1.430 (95% CI: 1.073 - 1.905; Table 3).
290 Regarding the effect of the food practices (Table 3), eating undercooked or raw meats or
291 unwashed produce were identified as well as risk factors. The poor handling of foods (mainly
292 “not washing hands before eating” or “using unwashed kitchen utensils”) was also found
293 associated with positive *Toxoplasma* serology in pregnant women (Table 1).

294

295 For most of the meta-analytical models reported in Tables 1, 2, and 3 the statistical tests
296 indicated the absence of potential significant publication bias. Exceptions were observed in
297 partitions related to food (Table 1), meat (Table 2), for mixed and pregnant population, and
298 practices of cooking meat (Table 3) (all populations). The funnel plots for food and meat in
299 the mixed population (Figure 6) evidences asymmetry at its basis, in an overestimation
300 manner, and underestimation at the top. The weight given in meta-analysis is lower in study
301 outcomes located at the basis than in those falling at the top of the funnel. The funnel plots for
302 food and meat in pregnant women, as for meat cooking practices, show little asymmetry that
303 may lead to some overestimation (Figure 6). The intra-class correlation I^2 indicates significant
304 low (<25%) to moderate (<50% or around 50%) remaining between-study heterogeneity in all

305 the data partitions. As Q or QE statistic was still significant, the moderator(s) already
306 considered in the meta-analysis models could not fully explain the between-study
307 heterogeneity (Gonzales-Barron et al., 2019).

308

309 **4. Discussion**

310 The measurement of seroprevalence is an indicator of *T. gondii* infection, but it does not
311 provide information on the time of infection. Besides, there may be a significant lapse of time
312 between contamination and the collection of information on the exposures of infected persons
313 (most often identified by serological research of IgG) during studies. Consequently, the
314 results are conditioned on the absence of any change in the respondents' exposures over time.
315 Cases could have been identified through serological tests whose performances (sensitivity
316 and/or specificity) are not necessarily equivalent between countries and over time. However,
317 we assume that these meta-analyses are not particularly biased (over or under-estimation) due
318 to those parameters. Furthermore, the origin of the infection (congenital or postnatally-
319 acquired) is not known in the primary studies included in this meta-analysis (case-control or
320 transversal studies). Nevertheless, the relative share of congenital infections in the
321 measurement of seroprevalence in children and adults is supposed to be low.

322

323 Considering the high prevalence of *Toxoplasma* infection globally, and the diversity of
324 pathways and sources of exposure to humans, the number of publications and investigated
325 risk factors is large and, most publications are recent. In comparison with the systematic
326 reviews of other foodborne pathogens of this special issue, this is the one with the highest
327 number of included case-control, cohort, and cross-sectional studies. As toxoplasmosis
328 outbreaks are rare, studying sporadic cases is the main way to identify potential sources, and
329 to make specific recommendations to populations at-risk such as pregnant women or
330 immunocompromised individuals. Then, our results are based most often on a considerable
331 number of outcomes and are in agreement with other published evidence.

332

333 Blood transfusion was identified as a source of toxoplasmosis in this meta-analysis, which
334 may seem at first surprising, but it has been reported as a risk factor in recent case-control
335 studies and molecular tools allow the detection of *Toxoplasma* DNA in blood samples (Saki *et*
336 *al.*, 2019). Blood transfusion is thought to be a rare source of transmission because the
337 duration of parasitemia during acute infection is brief but transmission by tachyzoites stage
338 is conceivable. Immunocompromised conditions were found to be associated with

339 *Toxoplasma gondii* infection in mixed population: this result is in agreement with a recent
340 meta-analysis (Wang *et al.*, 2017). However, its interpretation is not straightforward because
341 the serological methods could be less sensitive and specific in immunocompromised
342 individuals (Wang *et al.*, 2017).

343

344 As expected, exposure to cats appeared as a significant risk factor, which has long been
345 recognized in many case-control studies (Cook *et al.*, 2000). Felids, specifically the domestic
346 cat, have a central role as the source of infective oocysts; the implications of increasing cat
347 populations as a public health risk due to toxoplasmosis have been reviewed in Dabritz and
348 Conrad (2010). One consequence has been increased *T. gondii* oocyst dissemination to the
349 environment, associated with a higher risk of transmission to humans and animals. Significant
350 risk factors as “contact with other animals” are probably due to unhygienic contamination,
351 concerning environmental contamination (e.g. soil on the fur), and “contact with flies or
352 rodents” in children, could reflect indirect contamination. Although this risk factor is not often
353 studied, the role of flies and other pests has been identified as vectors by oocyst transport
354 (Frenkel *et al.* 1995). Environmental transmission by oocyst stage appears to be influential;
355 identified risk factors are contact with wastewater associated with poor hygiene, contact with
356 soil, farm environment that aggregates different risk factors not clearly defined, and
357 consumption of untreated or inadequately treated water.

358 For occupational exposure, farm animals exposure, and farm environment, more precise
359 definition about the raised animals, e.g., sheep vs. cattle vs. pig farming vs. mixed, or their
360 raising conditions, in future studies, could allow to break down those categories for further
361 meta-analysis investigation. Our results are in agreement with a systematic review of
362 outbreaks that showed that in 38 outbreaks (worldwide), 21% could be attributed to
363 contaminated waters and 26% to contact with contaminated soil (Meireles *et al.*, 2015).
364 Contamination of water and soils by oocysts was shown in different studies (Aubert and
365 Villena, 2009; Bahia-Oliveira, 2017; Bahia-Oliveira *et al.*, 2003; Shapiro *et al.*, 2019).

366

367 With regards to food consumption, undercooked meat, unwashed vegetables, raw milk, and
368 shellfish are risk factors for positive *Toxoplasma* serology. Those results are in accordance
369 with the analysis of published outbreaks, showing that raw or undercooked meat was the
370 origin of 44.7% of the outbreaks and raw vegetables of 5.3% (Meireles *et al.*, 2015).
371 Furthermore, our results are in line with the meta-analysis conducted by Belluco *et al.* (2018)
372 to assess the risk of humans developing acute *T. gondii* infection, due to the consumption of

373 undercooked beef, sheep (or meat), which were also found significant. In their study,
374 consumption of raw/undercooked pork, raw eggs, and unpasteurized milk were non-
375 significant risk factors. Concerning the role of meat, raw or undercooked meats were
376 identified as significant risk factors for toxoplasmosis. Within meats, with except for wild
377 boar, the type of meat found is consistent with the literature. Five publications, all conducted
378 in Mexico, identified wild boar meat as a risk factor (which is a low number of studies
379 compared to other risk factors). Game meat also stands out significantly. *T. gondii* has been
380 detected in sheep and lamb (Dubey, 2009; Halos *et al.*, 2010), cattle (Dubey 1986;1992;
381 Fromont *et al.*, 2009; Blaga *et al.*, 2019), pork (Djokic *et al.*, 2016; Foroutan *et al.*, 2019),
382 wild boar (Roqueplo *et al.*, 2017; Rostami *et al.*, 2017) and also other animals like poultry
383 (Tonouhewa, 2017; Guo *et al.*, 2016).

384

385 The identification of raw milk as a risk factor is based on 16 publications for the mixed
386 population and 27 for pregnant women. A recent review on milk consumption and
387 *Toxoplasma* infection reported mainly goat milk as a source of infection (Boughattas, 2017).
388 Moreover, *T. gondii* has been detected in raw milk from infected animals (Cisak *et al.*, 2017;
389 Vismarra *et al.*, 2017). Further studies should investigate the contamination in milk
390 (sheep/cows), and the spatial and temporal variability of contamination in farms and animals
391 could be better explored in the future. Shellfish (oysters, mussels, etc.) are identified from
392 four studies conducted in Taiwan (2), the United States (1), and the United Kingdom (1) only.
393 Shellfish contamination has been recently established (Cong *et al.*, 2017; Ghozzi *et al.*, 2017;
394 Coupe *et al.*, 2018). However, compared with meat-producing and poultry animals, research
395 on seafood species contaminated with *T. gondii* represents a relatively new field of study, and
396 harmonized methods of oocysts detection are lacking (Shapiro *et al.*, 2019).

397

398 Lack of hygiene, in particular, the lack of handwashing before eating or preparing a meal, was
399 also found to play a role in the acquisition of *Toxoplasma* infection. Produce, in particular
400 unwashed vegetables, was a significant risk factor which is expected considering the life cycle
401 of the parasite. The contamination of vegetables was explored in few studies, but data are
402 lacking in numerous countries due to the absence of standard detection methods for *T. gondii*
403 oocysts in these matrices (Shapiro *et al.*, 2019). The vast range of types of vegetables, not
404 described in the studies, could be better investigated (i.e., leaf or root vegetables).

405

406 **5. Conclusion**

407

408 The risk factors identified in this meta-analysis could complement those already established in
409 future sporadic case-control studies quantifying the attributable risk fraction: mollusks
410 (regarding the species), raw milk (goat/cows), vegetables (including the type of vegetable and
411 the preparation i.e., washing), game meat and drinking water. Furthermore, the development
412 of sensitive methods for the detection and isolation of *T. gondii* in these matrices is needed to
413 confirm the causal association revealed in epidemiological studies. In the context of the global
414 market of food and animals, it is essential to obtain information from an international meta-
415 analysis. This meta-analysis can be seen as a tool to identify emerging situations. The
416 transmission pathways need further investigations using longitudinal studies (to avoid
417 memory bias) and subtyping approaches (to differentiate between strain infectivity).
418 Information on the frequency or the duration of exposure could be further investigated.
419 Investigation of sporadic cases of infection remains essential for further understanding of
420 *Toxoplasma gondii* transmission.

421

422 **Acknowledgements**

423 The authors would like to thank ANSES staff and the members of the ANSES Working
424 Group on Source Attribution of Foodborne Diseases: Moez Sanaa, Laurence Watier, Jean
425 Christophe Augustin, Frédéric Carlin, Julie David, Philippe Fravallo, Laurent Guillier,
426 Nathalie Jourdan-Da Silva, Alexandre Leclercq, Lapo Mughini-Gras, Nicole Pavio.

427 U. Gonzales-Barron and V. Cadavez are grateful to the Foundation for Science and
428 Technology (FCT, Portugal) and FEDER under Programme PT2020 for the financial support
429 to CIMO (UID/AGR/00690/2019). U. Gonzales-Barron acknowledges the national funding by
430 FCT, P.I., through the Institutional Scientific Employment Program contract.

431

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621 **FIGURE CAPTIONS**

622 Figure 1: Flow chart of literature search for case-control, cohort and cross-sectional studies of
623 human sporadic toxoplasmosis

624

625 Figure 2: Forest plot of the association of blood transfusion with positive *Toxoplasma*
626 serology in pregnant women

627

628 Figure 3: Forest plot of the association of flies/rodents with positive *Toxoplasma* serology in
629 the mixed population

630

631 Figure 4: Forest plot of the association of consumption of dairy products with positive
632 *Toxoplasma* serology in the mixed population

633

634 Figure 5: Forest plot of the association of consumption of molluscs with positive *Toxoplasma*
635 serology in the mixed population

636

637 Figure 6: Funnel plots of meta-analyses investigating categorized risk factors (i.e., food in
638 mixed and pregnant population, meat in mixed and pregnant population, and practices of
639 cooking meat)

640

641 **LIST OF TABLES**

642 Table 1. Meta-analysis results on main (significant) risk factors for positive *Toxoplasma*
643 serology

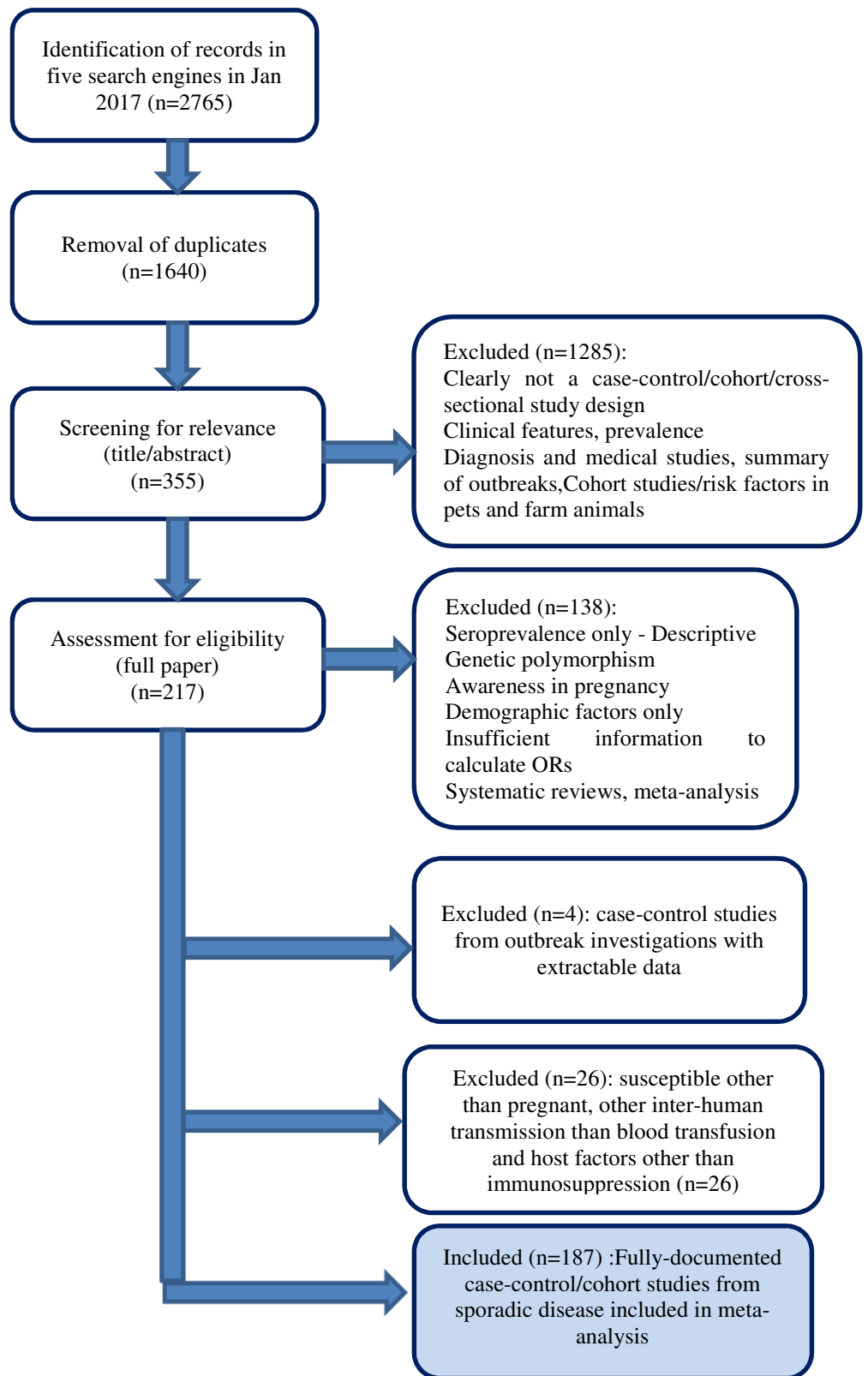
644 Table 2. Meta-analysis results on disaggregated risk factors for positive *Toxoplasma* serology

645 Table 3. Effect of poor handling on the pooled association between positive *Toxoplasma*
646 serology and consumption of meat, vegetables and dairy

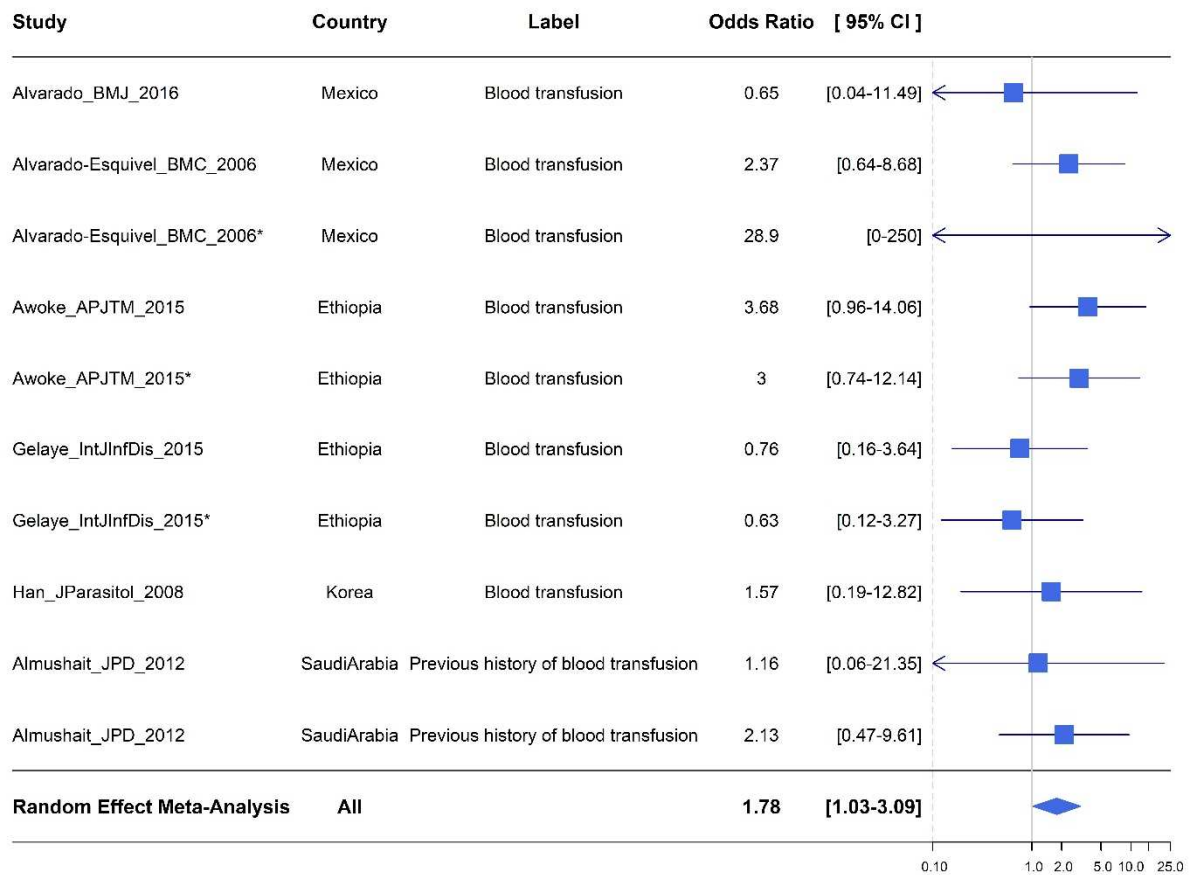
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692 Figure 1: Flow chart of literature search for case-control, cohort, or cross-sectional studies of
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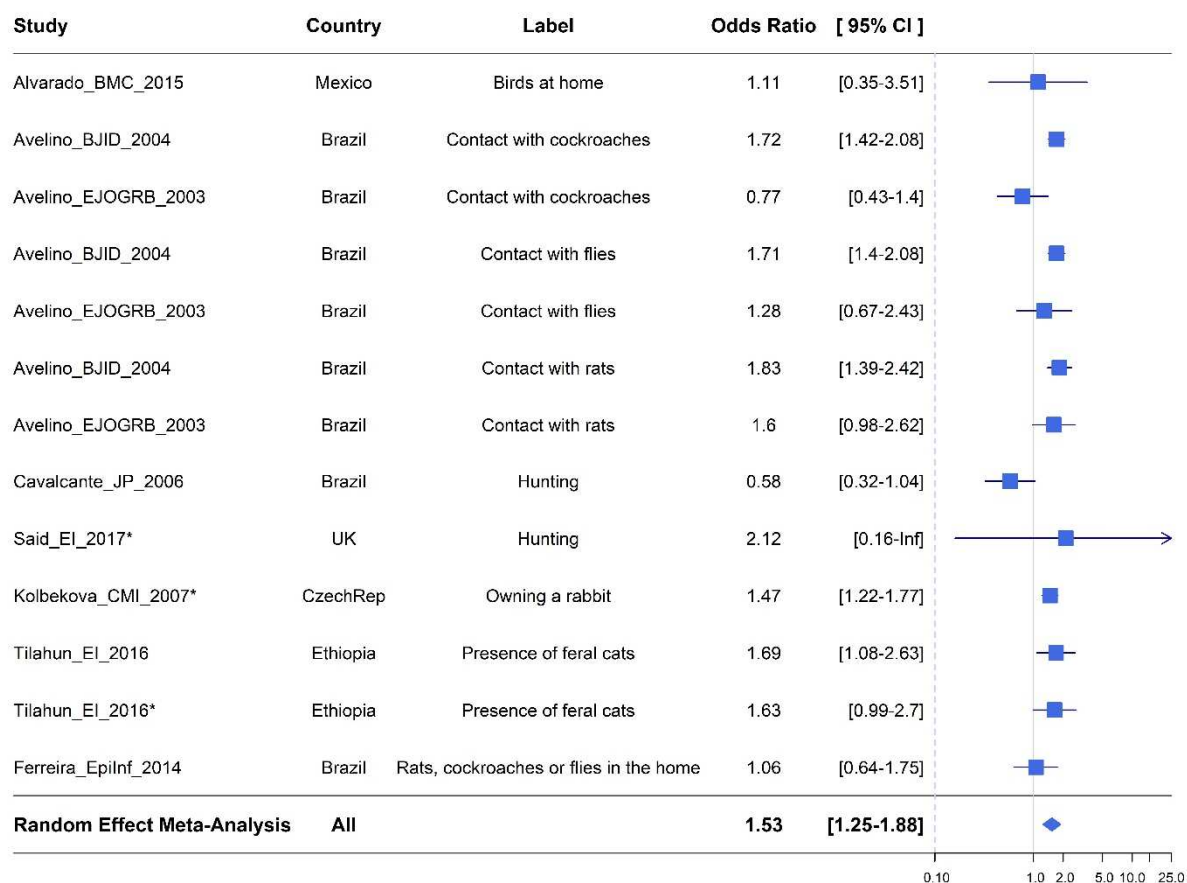


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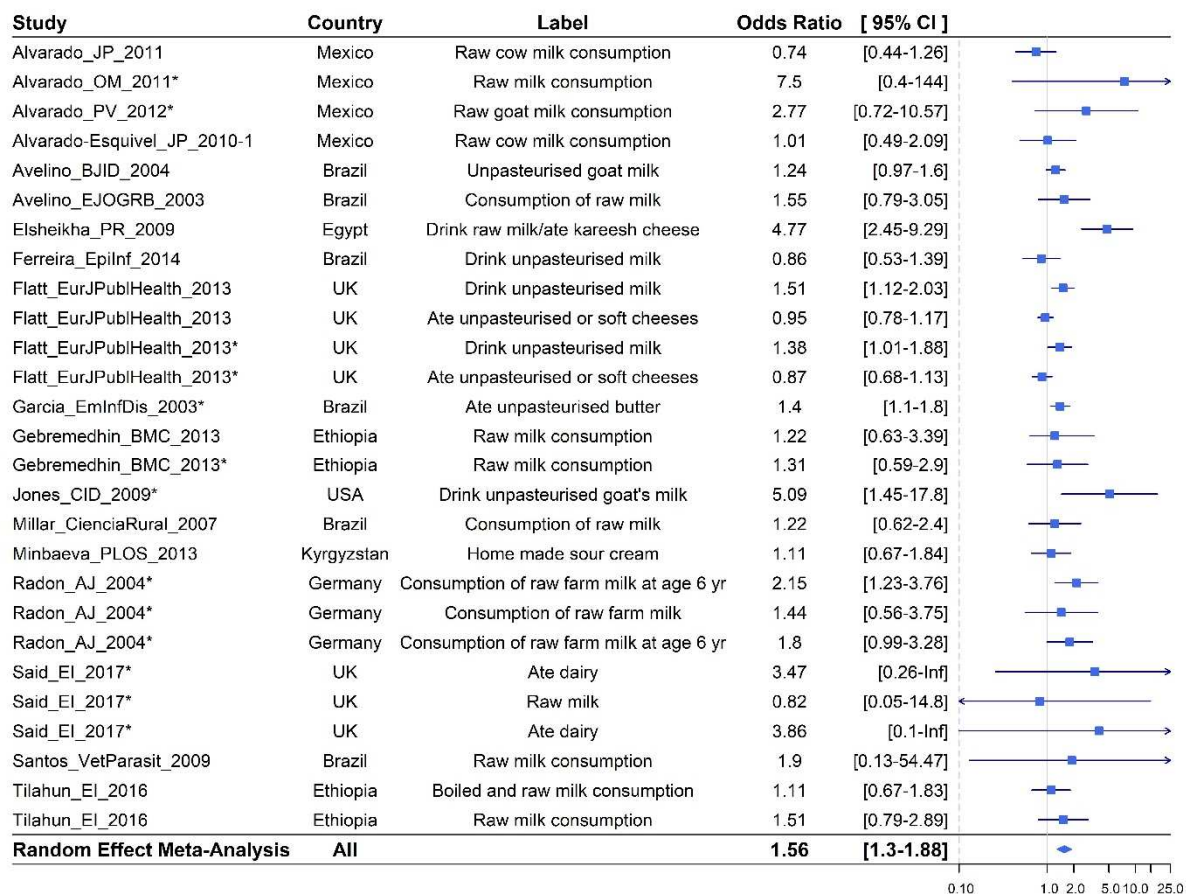
697 Figure 2: Forest plot of the association of blood transfusion with positive *Toxoplasma*

698 serology in pregnant women. (* adjusted OR as described in Gonzalez-Barron *et al.*, 2019)



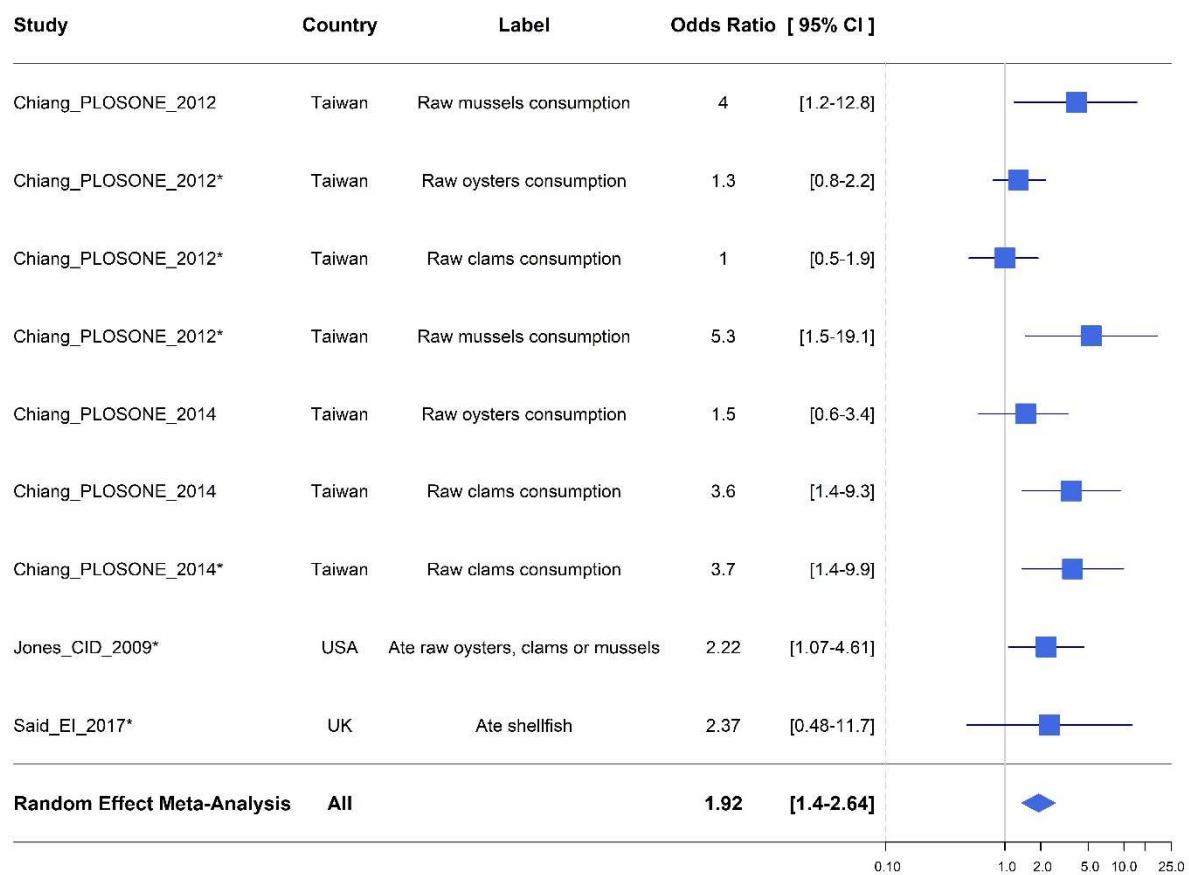
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700 Figure 3: Forest plot of the association of flies/rodents with positive *Toxoplasma* serology in
701 the mixed population. (* adjusted OR as described in Gonzalez-Barron *et al.*, 2019)



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703 Figure 4: Forest plot of the association of consumption of dairy products with positive
704 *Toxoplasma* serology in the mixed population. (* adjusted OR as described in Gonzalez-
705 Barron *et al.*, 2019)

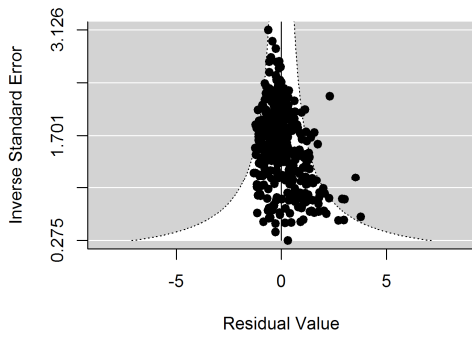


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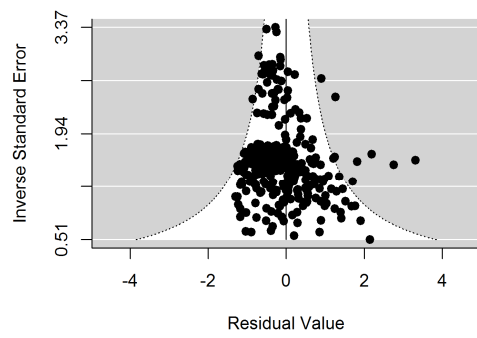
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 709 2019)

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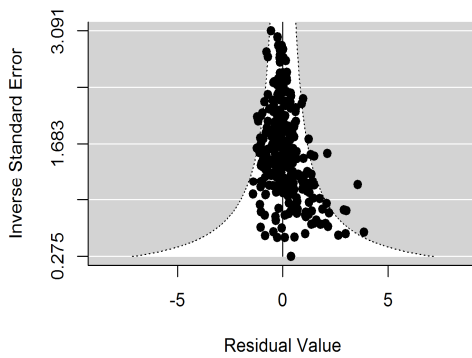
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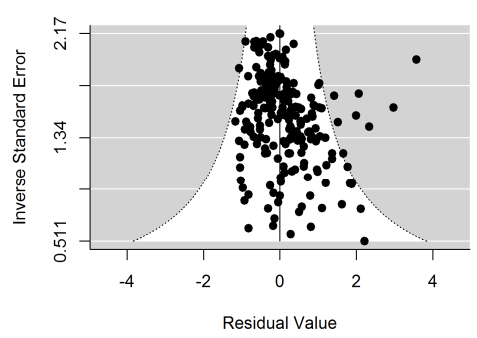
Food in the mixed population



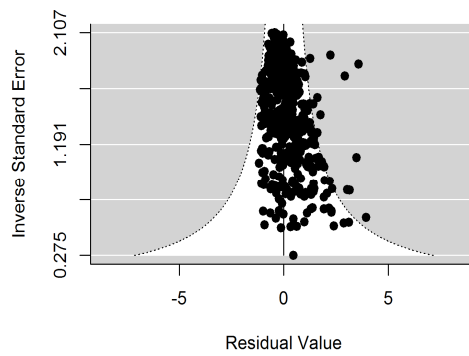
Food in pregnant women



Meat in the mixed population



Meat in pregnant women



Practices of cooking meat (“handling”)

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713 Figure 6: Funnel plots of meta-analyses investigating categorized risk factors (i.e., food in
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 715 cooking meat)

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720 Table 1. Meta-analysis results on main (significant) risk factors for positive *Toxoplasma* serology

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Population	Geographical area	Risk factor	Pooled OR [95% CI]	N/n*	p-val risk factor	Publication bias p-value	Points removed **	Heterogeneity analysis***
Travel								
Pregnant	All	Abroad	1.878 [1.284 - 2.746]	6/7	0.001	0.754	0	$\tau^2=0.083$ Q(df=6) = 11.08; p-val = 0.086 $s^2=0.380$ $I^2=17.94$
Host specific								
Mixed	All	Immuno-compromising conditions	2.407 [1.483 - 3.909]	14/32	0.001	0.188	0	$\tau^2=0.175$ QE(df=41) = 128.1; p-val < .0001 $s^2=0.955$ $I^2=15.451$
Pregnant	All	Blood Transfusion	1.785 [1.031 - 3.089]	6/10	0.039	0.063	0	$\tau^2=0.003$ QE(df=23) = 14.51; p-val = 0.911 $s^2=0.812$ $I^2=0.368$
Personal Hygiene								
All	All	Poor personal hygiene	2.023 [1.693 - 2.416]	3/9	<.0001	0.743	0	$\tau^2=0$ Q(df=8) = 6.596; p-val = 0.581 $s^2=0.241$ $I^2=0$
Animals								
Mixed(at)	All	Farm animals	1.482 [1.099 - 1.998]	6/8	0.009	0.176	0	$\tau^2=0.801$ QE(df=218)= 838.7; p-val < .0001 $s^2=0.555$ $I^2=59.1$
		Occupational	2.035 [1.641 - 2.522]	20/65	<.0001			
		Pets	1.759 [1.496 - 2.067]	67/137	<.0001			
		flies/rodents	1.534 [1.249 - 1.882]	8/13	<.0001			
Pregnant	All	Occupational	1.557 [1.245 - 1.948]	8/9	0.0001	0.214	0	$\tau^2=0.163$ QE(df=198)= 511.4; p-val < .0001 $s^2=0.553$ $I^2=22.75$
		Pets	1.536 [1.374 - 1.717]	71/176	<.0001			
		flies/rodents	1.470 [1.133 - 1.908]	4/6	0.004			
Children	All	Pets	1.634 [1.331 - 2.005]	14/74	<.0001	0.234	0	$\tau^2=0.539$ QE(df=82) = 413.7; p-val < .0001 $s^2=0.356$ $I^2=60.27$
		Wild	1.435 [1.052 - 1.959]	3/6	0.023			

Environment								
Mixed	Africa removed (22 ORs)	Untreated drinking water	1.429 [1.217 - 1.677]	32/64	<.0001	0.061	0	I ² =0.588 QE(df=197)= 709.0;p-val < .0001 s ² =0.359 I ² = 62.03
		Farm environment	1.363 [1.146 - 1.621]	37/62	0.001			
		Playground	1.656 [1.403 - 1.953]	41/66	<.0001			
		Waste water****	1.523 [1.047 - 2.214]	4/6	0.028			
Pregnant	All	Untreated drinking water	1.487 [1.279 - 1.729]	34/54	<.0001	0.187	0	I ² =1.071 QE(df=189)= 534.4; p-val < .0001 s ² =0.539 I ² =66.50
		Farm environment	1.804 [1.465 - 2.221]	32/52	<.0001			
		Playground	1.462 [1.319 - 1.621]	47/74	<.0001			
		Waste water	1.863 [1.162 - 2.986]	7/11	0.010			
Children	Oceania (1 OR excluded) and Africa excluded (6 OR excluded)	Untreated drinking water	1.403 [1.215 - 1.620]	2/23	<.0001	0.947	0	I ² =0.178 QE(df=47) = 64.15;p-val = 0.045 s ² =0.203 I ² =46.87
		Farm environment	2.642 [1.768 - 3.946]	3/7	<.0001			
		Waste water	1.802 [1.228 - 2.645]	2/5	0.003			
Food								
Mixed	Oceania excluded (1 OR excluded)	Dairy	1.563 [1.298 -1.882]	18/27	<.0001	0.031	4	I ² =2.212 QE(df = 396) = 1146, p-val < .0001 ; s ² =0.669 I ² =76.7569
		Meat	1.761 [1.570 - 1.974]	66/287	<.0001			
		Produce	1.872 [1.539 - 2.276]	37/58	<.0001			
		Seafood	1.702 [1.332 - 2.176]	4/12	<.0001			
Pregnant	All	Dairy	1.521 [1.116 - 2.073]	28/44	0.008	0.002	0	I ² =1.044 QE(df=36) = 1368; p-val < .0001 s ² =0.459 I ² =69.46
		Meat	1.960 [1.472 - 2.610]	65/241	<.0001			
		Produce	1.651 [1.267 - 2.151]	34/64	0.001			
Poor handling								
Pregnant	All	Poor (no handwashing before eating or cooking, no washing knife)	2.000 [1.598 - 2.504]	19/35	<.0001	0.599	0	I ² =0.378 QE(df=45) = 84.05; p-val = 0.001 s ² =0.489 I ² =43.607

722 *N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual
723 heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2). **** including non-compliant toilets or lack of toilets in the main dwelling; (at) the analysis type is significant: results are
724 given for multivariate estimates

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727 Table 2. Meta-analysis results on disaggregated risk factors for positive *Toxoplasma* serology

Main risk factor	Population	Geographical area	Specific class or specific population	Pooled OR [95% CI]	N/n*	Risk factor p-val	Publication bias p-val	Points removed**	Heterogeneity analysis***
Animals									
Cat	All	All	Mixed	1.711 [1.463 - 2.001]	63/112	<.0001	0.465	0	I ² =0.607 QE(df=311) = 1191;p-val < .0001 I ² =52.37 s ² =0.552
			Pregnant	1.631 [1.445 - 1.841]	70/150	<.0001			
			Children	1.653 [1.328 - 2.057]	13/52	<.0001			
Food									
Meat	Mixed	Oceania excluded (1 OR excluded)	Other red meats	1.897 [1.480 - 2.431]	23/48	<.0001	0.037	8	I ² = 1.499 QE(df=274)= 668.8;p-val < .0001 s ² =0.717 I ² =67.63
			Pork	2.114 [1.411 - 3.169]	15/23	0.001			
			Others	1.734 [1.544 - 1.947]	54/145	<.0001			
			Poultry	1.623 [1.147 - 2.297]	11/15	0.006			
			Processed meat	1.365 [1.075 - 1.733]	13/26	0.011			
			Beef	1.635 [1.170 - 2.285]	15/23	0.004			
Meat	Pregnant	All	Other red meats	1.822 [1.279 - 2.595]	11/33	0.001	0.002	0	I ² =1.608 QE(df=235)= 996.1;p-val < .0001 s ² =0.579 I ² =73.50
			Others	1.592 [1.354 - 1.871]	60/106	<.0001			
			Poultry	1.514 [1.130 - 2.028]	11/19	0.005			
			Processed meat	1.532 [1.201 - 1.953]	9/27	0.001			
			Beef	2.052 [1.576 - 2.672]	11/29	<.0001			
Other red meats	Pregnant	All	Lamb meat	1.832 [1.148 - 2.922]	14/4	0.011	0.260	0	I ² =0.762 QE(df=19) = 41.75;p-val = 0.002 s ² =0.754 I ² =50.27
Other red meats	Mixed	All	Lamb meat	2.404 [1.189 - 4.859]	5/6	0.015	0.096	2	I ² =0.859 QE(df=42) = 66.08;p-val = 0.010 s ² =0.633 I ² =57.615
			Boar meat	2.487 [1.814 - 3.409]	5/8	<.0001			
			Goat meat	1.667 [1.212 - 2.294]	7/8	0.002			
			Venison/ram/horse meat	1.566 [1.221 - 2.008]	9/12	0.001			
Produce	Mixed	All	Vegetables	1.866 [1.491 - 2.335]	33/52	<.0001	0.879	2	I ² =0.297 QE(df=56) = 198.6;p-val < .0001 s ² =0.522 I ² =36.29
Produce	Pregnant	All	Vegetables	1.372 [1.198 - 1.571]	34/57	<.0001	0.784	0	I ² =0.145 QE(df=62) = 124.3; p-val < .0001 ; s ² =0.234 I ² =38.32
Seafood	All	All	Mollusks	1.917 [1.395 - 2.636]	4/9	<.0001	0.087	0	I ² =0.019

